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Experience with targeted next generation sequencing for the care of lung cancer: insights into promises and limitations of genomic oncology in day-to-day practice

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Abstract

Introduction—Tumor genotyping using single gene assays (SGAs) is standard practice in advanced non-small-cell lung cancer (NSCLC). We evaluated how the introduction of next generation sequencing (NGS) into day-to-day clinical practice altered therapeutic decision-making.

Methods—Clinicopathologic data, tumor genotype, and clinical decisions were retrospectively compiled over 6 months following introduction of NGS assay use at our institution in 82 patient-tumor samples (7 by primary NGS, 22 by sequential SGAs followed by NGS, and 53 by SGAs).

Results—SGAs identified abnormalities in 34 samples, and all patients with advanced *EGFR*-mutated or *ALK*-rearranged tumors received approved tyrosine kinase inhibitors (TKIs) or were consented for clinical trials. NGS was more commonly requested for *EGFR*, *ALK*, and *KRAS*-negative tumors ($p<0.0001$). NGS was successful in 24/29 (82.7%) tumors. Of 17 adenocarcinomas (ACs), 11 (7 from patients with ≤15 pack-years of smoking) had abnormalities in a known driver oncogene. This led to a change in decision-making in 8 patients, trial consideration in 6, and off-label TKI use in 2. Of 7 squamous cell (SC) carcinomas, 1 had a driver

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CONFLICT OF INTEREST STATEMENT

Daniel B. Costa has received consulting fees from Pfizer and honoraria fromBoehringerIngelheim.
Deepa Rangachari, Paul A. VanderLaan, Xiuning Le, Erik Folch, Michael S. Kent, Sidharta P. Gangadharan, Adnan Majid, Richard L. Haspel, Loren J. Joseph and Mark S. Huberman have no conflicts to disclose.
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aberration (*FGFR1*); 6 had other genomic events (all with *TP53* mutations). In no cases were clinical decisions altered (p=0.0538 when compared to ACs).

Conclusions—Targeted NGS can identify a significant number of therapeutically-relevant driver events in lung ACs; particularly in never or light smokers. For SC lung cancers, NGS is less likely to alter current practice. Further research into the cost effectiveness and optimal use of NGS and improved provider training in genomic oncology are warranted.

Keywords

Non-small-cell lung cancer; next generation sequencing; genotype; EGFR; ALK; driver oncogene; MET; ROS1; MET; adenocarcinoma; squamous cell carcinomas

1. INTRODUCTION

The management of advanced non-small-cell lung cancer (NSCLC) is increasingly directed by knowledge of tumor genotype. Expert groups like the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), the Association for Molecular Pathology (AMP), and the American Society of Clinical Oncology (ASCO) now endorse routine testing for epidermal growth factor receptor (*EGFR*) mutations or anaplastic lymphoma kinase (*ALK*) rearrangements using rapid single gene assays (SGAs) (1-3). Knowledge of these predictive biomarkers has permitted selective application of tyrosine kinase inhibitors (TKIs) (1-3), with *EGFR* and *ALK* TKIs having gained approval from the U.S. Food and Drug Administration (FDA) on the basis of these genomic features (4-9).

The genetic landscape of NSCLC is complex. Oncogenic and/or therapeutically-relevant genomic aberrations include: mutations, amplifications, deletions, and rearrangements/ fusions. It is now well established that a significant proportion of lung adenocarcinomas (ACs) harbor mutations in driver oncogenes that can augment “sustained proliferative signaling” - a hallmark feature of tumorigenesis. These include mutations in: v-ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*), Raf murine sarcoma viral oncogene homolog B1 (*BRAF*), V-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*), rearranged during transfection (*RET*), c-ros oncogene 1 (*ROS1*), and neurotrophic tyrosine kinase receptor type 1 (*NTRK1*), among others (10). Squamous cell (SC) lung cancers may instead harbor genomic changes involving: fibroblast growth factor receptor (*FGFR*) 1/2/3/4, phosphatidylinositol-3-kinase catalytic subunit alpha1 (*PIK3CA*), and discoidin domain-containing receptor 2 (*DDR2*), among others (10, 11).

Technological advances have led to the introduction of next generation sequencing (NGS) platforms into the thoracic oncology clinic (10). As opposed to SGAs, NGS permits massive parallel sequencing that affords maximal tumor genomic assessment while using precious tumor samples sparingly (12). These NGS assays offered by Clinical Laboratory Improvement Amendments (CLIA)-certified commercial or academic vendors are usually only feasible when based on a targeted panel of genes (i.e. targeted NGS) that select for the most readily targetable alterations. Many of these NGS assays, especially whole genome and whole exome, require substantial nucleic acid input (250ng-1ug), though some have been

optimized to allow results from lower concentrations of deoxyribonucleic acid (DNA) and more recently ribonucleic acid (RNA) from formalin-fixed paraffin-embedded (FFPE) specimens or cytology specimens (12-14).

The feasibility and applicability of NGS in day-to-day clinical practice—as opposed to use in research settings alone—has not been well vetted in the literature to date. We therefore evaluated how the introduction of NGS assays into daily practice altered therapeutic decision-making for a cohort of NSCLCs treated by a multidisciplinary Thoracic Oncology team at this National Cancer Institute (NCI)-affiliated cancer center. In this cohort, NGS was applied as part of routine practice and not in parallel with other academic/commercial efforts, such as assay development or clinical trial screening.

2. METHODS

2.1 Cohort selection and data collection

Patients seen at Beth Israel Deaconess Medical Center (BIDMC, a member of the Dana-Farber/Harvard Cancer Center) with a diagnosis of NSCLC and whose tumors were submitted for either SGA or NGS were identified through an ongoing Institutional Review Board-approved study (15, 16). Patient inclusion was restricted from May 1st to October 31st, 2014 (the 6-month interval since introduction of NGS in clinical NSCLC specimens). Clinical, pathologic, radiographic, and tumor genotyping parameters were collected by retrospective chart extraction and managed using REDCap electronic data capture tools hosted at BIDMC. A review of clinical documentation, clinical trial screening/consent, and anti-cancer therapies administered allowed for determination of clinical decision-making.

2.2 Tumor genotyping

Following routine pathologic diagnosis of NSCLC, tumor material (from surgical specimens, core needle biopsies or cell aspirates/concentrates) in FFPE tissue blocks was submitted for genomic analysis. SGAs (*EGFR* exon 18-21 mutation analysis, *KRAS* codon 12 and 13 mutation analysis, *ALK* fluorescence in situ hybridization [FISH], *ROS1* FISH) and NGS were performed as previously described (13-17). Three different NGS assays were used. The first two were performed by an academic medical center (Massachusetts General Hospital; Boston, MA) using an anchored multiplex polymerase chain reaction (AMP) assay that employs a targeted sequencing strategy (13). The first AMP assay (SNaPshot-NGS-V1) evaluates single nucleotide variants (SNV) and insertions/deletions (indels) in genomic DNA using NGS targeting 39 putative oncogenes and tumor suppressor genes (13); this assay was used in 22 of the study cases. The second AMP assay (ALK, RET, ROS1 NGS Gene Fusion Assay) evaluates fusion transcript detection for *ALK*, *ROS1* and *RET* using genomic RNA (13); this assay was used in 6 of the study cases. The third NGS assay (FoundationOne, Foundation Medicine; Cambridge, MA) interrogates 315 genes as well as introns of 28 genes involved in rearrangements using massively parallel DNA sequencing to characterize base substitutions, short indels, copy number alterations, and selected fusions (14); this assay was used in 2 of the study cases. A CLIA-certified single gene *FGFR1* FISH test (Massachusetts General Hospital; Boston, MA) to evaluate copy number of *FGFR1* (17) was used in addition to NGS in SC carcinomas; this assay was used in 5 of the study cases.

2.3 Statistical methods

Fisher's exact test was used to compare categorical variables. All p-values reported were two-sided.

3. RESULTS

3.1 Patient and tumor characteristics

Table 1 illustrates baseline patient and tumor characteristics. The cohort comprised 82 patients, most of whom had stage IV/recurrent disease (72.0%) and AC histology (90.2%).

3.2 SGAs for EGFR/ALK/KRAS/ROS1 and clinical decisions

Figure 1 depicts the clinical use and outcomes of genomic analyses in the 82 patient-tumor samples. SGAs were ordered in 75 tumors. Analyses for abnormalities in *EGFR*, *ALK*, *KRAS*, and *ROS1* were successful in: 94.6% (71/75), 96% (72/75), 94.4% (68/72), and 79.7% (55/69), respectively. The increased failure rate of *ROS1* testing is noted, raising the possibility of technical issues with the FISH test (rather than inherent lack of tumor material). Of successfully genotyped tumors, abnormalities in *EGFR, ALK, KRAS, and ROS1* were found in: 14.0% (10/71), 4.1% (3/72), 30.8% (21/68), and 0% (0/55), respectively. Abnormalities in *EGFR*, *KRAS*, and *ALK* were mutually exclusive—except in one case with a concomitant *EGFR* exon 19 deletion and positive *ALK* FISH identified on the same sample.

A total of 10 patients with tumors harboring *EGFR* mutations were identified. Of these, 8 with metastatic AC received an FDA-approved EGFR TKI (erlotinib), with 3 of the 8 consenting for a clinical trial of erlotinib (www.clinicaltrials.gov: NCT00997334). One patient with stage III disease consented for a clinical trial of adjuvant afatinib (NCT01746251). One patient with stage III disease received concurrent chemoradiation, as per evidence-based guidelines.

All 3 patients with *ALK*-rearranged ACs received an FDA-approved ALK TKI: one received crizotinib, and 2 were consented for a clinical trial evaluating crizotinib versus alectinib (NCT02075840).

The 21 patients with *KRAS*-mutated ACs received standard therapies as per expert guidelines (3). Of the 16 patients with stage IV disease, 2 were eligible for and were offered a clinical trial (NCT01933932) following progression on standard platinum-doublet therapy.

35 patients had tumors that were negative for abnormalities in *EGFR*, *ALK*, and *KRAS*. None of these patients were offered off-label TKIs, but 3 of these patients consented for clinical trials using cytotoxic chemotherapy as a backbone.

3.3 Use of NGS assays and clinical decisions

NGS assays were ordered in 29 tumors, corresponding to 35.3% of the original cohort. 22 NGS requests (21 ACs, 1 SC carcinoma) were made for tumors that had previously undergone first-pass SGAs. For the remaining 7 NGS requests (1 AC, 6 SC carcinomas), NGS was the lone genomic test requested (Figure 1B). Of the 22 NGS assays that were

requested following initial SGAs, nearly all (21/22, 95.4%) came from tumors that had previously tested negative for abnormalities in *EGFR*, *ALK*, and *KRAS*. Conversely, the majority (21/35, 60%) of tumors testing negative for *EGFR*, *ALK*, and *KRAS* on initial SGAs were subsequently sent for NGS (Figure 1A). There were no observed discrepancies between NGS and SGA results. Of the 29 total tumors submitted for NGS, 24 (82.7%) had successful results. The failure rate was higher if the sample had been previously submitted for SGAs (5/22, 22.7%), as compared to a failure rate of 0% (0/7) when NGS was the initial genomic test (p=0.2965).

Of the 24 successful samples undergoing NGS, most (17/24, 70.8%) were of AC histology, the majority of which (11/17, 64.7%) harbored a well-established driver oncogene: 1 *EGFR*-mutated, 2 *ERBB2*-mutated, 1 *ROS1*-rearranged, 1 *RET*-rearranged, 2 *KRAS*-mutated, 1 *MET*-amplified tumor, 2 *MET*-exon 14 splicing mutated, and 1 *MAP2K1*-mutated tumor; concurrent *TP53* mutations were seen in 40% of cases. Of the 6 tumors without an identifiable principal oncogene mutation, *TP53* mutations were most common (66.6%). Details of individual tumor genotyping results and clinical decisions are listed in Table 2.

Clinical decisions were impacted by NGS results in 8/17 (47.0%) ACs. A total of 6 patients were considered for clinical trials available at our institution on the basis of findings from NGS: 4 consented for a clinical trial of an oncogene-specific TKI, and 2 were offered a genotype-specific trial upon subsequent progression. One patient with an *ERBB2*-mutated AC received off-label afatinib (a clinical trial for this genotype had closed to accrual). Another patient with a *MET* exon 14 splicing mutation was offered off-label crizotinib at first progression. While not FDA-approved for the latter two indications, these TKIs are acknowledged as targeted therapies for consideration as per the National Comprehensive Cancer Network (NCCN) guidelines for NSCLC (3).

The results of NGS assays were of lesser therapeutic consequence in the 7 SC carcinomas, where all tumors harbored *TP53* mutations but lacked the prevalent/principal driver oncogenes observed in ACs (1/7 or 14.2% vs. 10/17 or 58.8% in SC vs. AC, respectively; p=0.0778). Only 1 case of borderline *FGFR1* amplification was identified, but this did not lead to a change in clinical decision-making as the clinical trial of an FGFR inhibitor (NCT01703481) available at our institution at the time was not applicable for this patient; this patient received concurrent chemoradiation as per expert guidelines. Therefore, in none of the cases of SC carcinoma were clinical decisions altered by NGS results—a proportion that was notably lower than that observed in those patients with AC (0% or 0/7 vs. 47.0% or 8/17 for SC vs. AC, respectively; p=0.0538). The therapeutic utility of tumor genotyping stratified by histology is indicated in Figure 2 and Table 2.

3.4 Alterations in driver oncogenes in lung ACs from never or light smokers

Driver oncogenes - for which TKIs are approved or have some level of clinical evidence (i.e. *EGFR*, *ALK*, *ROS1*, *RET*, *ERBB2*, *MET*) - identified by combined SGAs and/or NGS (Figure 2) were significantly more frequent in patients with a ≤ 15 pack-year tobacco history (18/27, 66.6%) than in those with > 15 pack-years of tobacco use (3/42, 7.1%; p<0.0001). *KRAS* mutations were more frequent in the group with > 15 pack-years of tobacco use (17/42 or 40.4% vs. 5/27 or 18.5%, respectively; p=0.0682).

4. DISCUSSION

The care of advanced NSCLC has evolved dramatically in the last decade, owing to the evolution of targeted, biologically pertinent therapies offered on the basis of vetted genomic biomarkers. When used in these genotype-specific settings, targeted therapies have afforded consistent improvements in progression-free survival and quality of life, as compared to conventional cytotoxic agents (4-10). Unsurprisingly, oncology patients and providers in academic and community settings alike are increasingly aware of, desire, and are incumbent to obtain timely and accurate tumor genomic testing to help guide therapeutic decision-making (18, 19). It is also now apparent that the genomic landscape of NSCLC extends beyond *EGFR* and *ALK* alone, with potentially relevant aberrations also found in: *KRAS*, *BRAF*, *ERBB2*, *RET*, *ROS1*, *NTRK1*, *FGFR* 1/2/3/4, *PIK3CA*, and *DDR2*, among others (10, 11).

4.1 Outcomes of NGS use in NSCLC by other groups

The clinical utility of comprehensive genomic analyses of lung ACs has been recently highlighted by the Lung Cancer Mutation Consortium (LCMC), which analyzed 10 potential genomic drivers from tumor specimens obtained from academic centers nationwide (20). An oncogenic driver was found in 64% of tumors; these results were subsequently used to select a precision therapy or clinical trial in 28% of cases. Notably, median survival was improved for those patients whose tumors harbored an abnormality amenable to a genotype-directed therapy (20). The Washington University lung cancer program recently published their experience with an internally-developed 23-gene NGS assay (21). A 45% assay failure rate was noted; however, NGS afforded actionable information in 46% of successfully assayed NSCLCs, and a targeted therapy was instituted in 11% of genotyped tumors (21). Similarly, colleagues at Memorial Sloan Kettering used hybrid-capture-based NGS testing (FoundationOne) following serial negative SGAs in patients with AC and light/no (≤15 pack-year) tobacco use (22). 34% of patients were excluded on the basis of tissue exhaustion from prior testing. Actionable genomic changes were identified in 39% of patients (using targeted therapies on/off study) (22). We are unaware of other peer-reviewed publications detailing the clinical application of NGS in day-to-day practice in a multidisciplinary NSCLC clinic.

4.2 NGS in EGFR, ALK, KRAS-wild type tumors

In the cohort presented here, NGS was used in greater than one-third of cases and in a rationally selected subset with tumors most likely to harbor changes that may render genotype-directed therapy of use, i.e.: patients without other common driver mutations (*EGFR, ALK*, and *KRAS*) and/or those with light/no tobacco use (≤15 pack-years). This cohort highlights the application of NGS in routine clinical practice and not in parallel with other efforts (i.e. assay development or clinical trial screening) (20-22). Therefore, it serves as an instructive platform for assessing how this technology is being adopted, implemented, and applied in daily care.

4.3 Pearls and pitfalls of NGS use

Important limitations of this analysis include: its retrospective nature, the limited sample size, utilization of archival tissue, and absence of direct involvement in NGS analysis/interpretation. Moreover, we currently have no pre-specified algorithm to direct obtaining and integrating genomic results into patient care, beyond application of approved targeted therapies in selected populations.

NGS was successfully executed in the vast majority of submitted samples (>80%). As observed by our group and others, driver oncogenes with known therapeutic potential were more readily identified in those with AC vs. SC histology. NGS of ACs impacted real-world decision-making in nearly half of cases, enabling genotype-specific therapies. In most ACs in which a driver mutation was identified and a relevant clinical trial available/appropriate, enrollment was offered. We favor this strategy for integrating genomic data into patient care, so as to drive participation in genotype-directed trials and facilitate rational and rigorous approval of novel therapies and targeted applications. During our data collection period, our center had active protocols for tumors with aberrations in *EGFR*, *ALK*, *ROS1*, *MET*, *KRAS* and *ERBB2*.

4.4 NGS use in SC carcinomas

Genomic results (NGS or SGAs) for SC carcinomas did not lead to practice changes in our cohort. The number of cases analyzed was small, and additional numbers would have likely revealed tumors with *PI3KCA*, *DDR2*, and *FGFR* aberrations; these have been described by other groups as potentially targetable in preclinical models (10, 11). However, the absence of known bona fide driver oncogenes in the majority of SC carcinomas (10) has made the evolution of targeted therapies for this histologic subtype much slower. To date, no TKI is approved by regulatory agencies or recommended by the NCCN for SC carcinomas (3). This unmet clinical need has led to development of novel biomarker-driven trials. The National Clinical Trials Network (NCTN) has recently started the LUNG-Map/S1400 (NCT02154490) trial to address this need (http://www.lung-map.org/). This is a multi-armed randomized phase II/III trial emphasizing matched gene-drug strategies using the FoundationOne platform for NGS. If LUNG-Map is successful, then NGS and novel targeted drugs may become part of the standard approach to managing SC lung cancers, as well.

4.5 Optimizing NGS use in day-to-day practice

It is speculative to consider whether if used as a sole method, NGS may have allowed for detection of all clinically-relevant genomic alterations, with a higher success rate, and without the time, effort, cost, and tissue exhaustion associated with sequential testing. As have others, we note that there was a trend toward more frequent NGS failure rates in samples where SGAs preceded. These factors underscore the critical importance of timely and effective collaboration between members of the multidisciplinary team to obtain, select, and retain optimal specimen(s) for effective testing. Enthusiasm for the routine use of up-front NGS should be tempered by consideration of the financial, medical, and psychosocial costs and cost effectiveness of this approach. This includes consideration of “costs” associated with the assay itself, subsequent anti-cancer medication(s), additional tissue

acquisition procedures, therapeutic delays pending tissue testing, and inadequately known benefits/risks for the patient. Such efforts should occur in parallel with the development and application of these genomic platforms. It is an urgent need to establish the optimal use of genomic testing (i.e. sequential SGAs versus NGS) in the day-to-day care of NSCLC patients.

The evolution of genotype-directed cancer care also has implications for the education of medical professionals. A recent publication testifies to this unmet need: in a study of 160 faculty members at a well-known academic cancer center, higher “genomic confidence” was associated with a higher likelihood that a provider would obtain and apply genomic data to patient care; 22% of surveyed cancer providers indicated low confidence in their genomic knowledge (19). Targeted areas for genomic oncology curricula should include: methodologies for genomic assessment (i.e. understanding what methods are specifically utilized by commercial/institutional genomic assay vendors); optimal/adequate specimen selection; utilization of validated resources for interpretation of results; understanding the therapeutic implications—or lack thereof—of a given finding; obtaining informed consent for testing from patients; and discussing results of testing with patients—including findings of uncertain significance. In recognition of this evolving and increasingly apparent educational need, colleagues have already started to take systematic approaches to educate trainees in this important domain (23-25).

5. CONCLUSIONS

In summary, we report the patterns of use and implications of NGS testing in an academic thoracic oncology clinic. Sequential single gene and targeted NGS assays can identify a significant number of confirmed driver events in lung ACs for which targeted therapies are either already available or in development. NGS is particularly impactful in never/light smokers and in whom first pass SGAs do not identify any targetable abnormalities, as this subset is likely to be enriched with oncogenic targets that are of therapeutic relevance. In SC lung cancers, however, the impact of NGS in standard practice remains unclear, and innovative biomarker-driven trials have commenced to address this unmet need. The growth of platforms that permit rapid tumor genotyping has fueled a furtive interest in the development and application of personalized approaches in cancer therapeutics. Comprehensive first line genotyping of NSCLCs using NGS is therefore an attractive extension of these efforts. However, future research into the cost and relative impact/effectiveness of this approach, as well as improved training of medical professionals in the practice of genomic oncology, are warranted to maximize this strategy of care.

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CLINICAL PRACTICE POINTS

- Tumor genotyping using single gene assays for *EGFR* mutation and *ALK* rearrangementare standard practice in advanced non-small-cell lung cancer;

- Targeted next generation sequencing can identify a significant number of therapeutically-relevant driver events in lung adenocarcinoma; particularly in never or light smokers;

- The introduction of next generation sequencing/comprehensive molecular profiling into day-to-day clinical practice altered therapeutic decision-making and increased the number of cases that can benefit from precision oncology;

- Further research into the cost effectiveness and optimal use of next generation sequencing and improved provider training in genomic oncology are warranted.

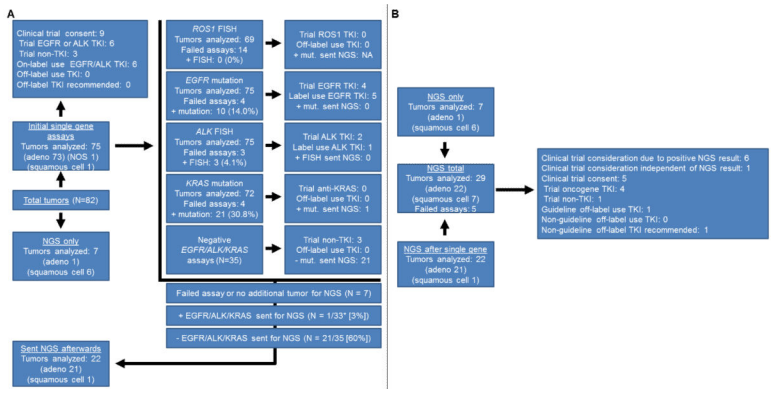

Figure 1.

Flow chart of genomic analyses over a 6-month period. A.) Single gene assays: results, clinical decisions and trial evaluation. B.) Next generation sequencing (NGS) assays: results, clinical decisions and trial evaluation.

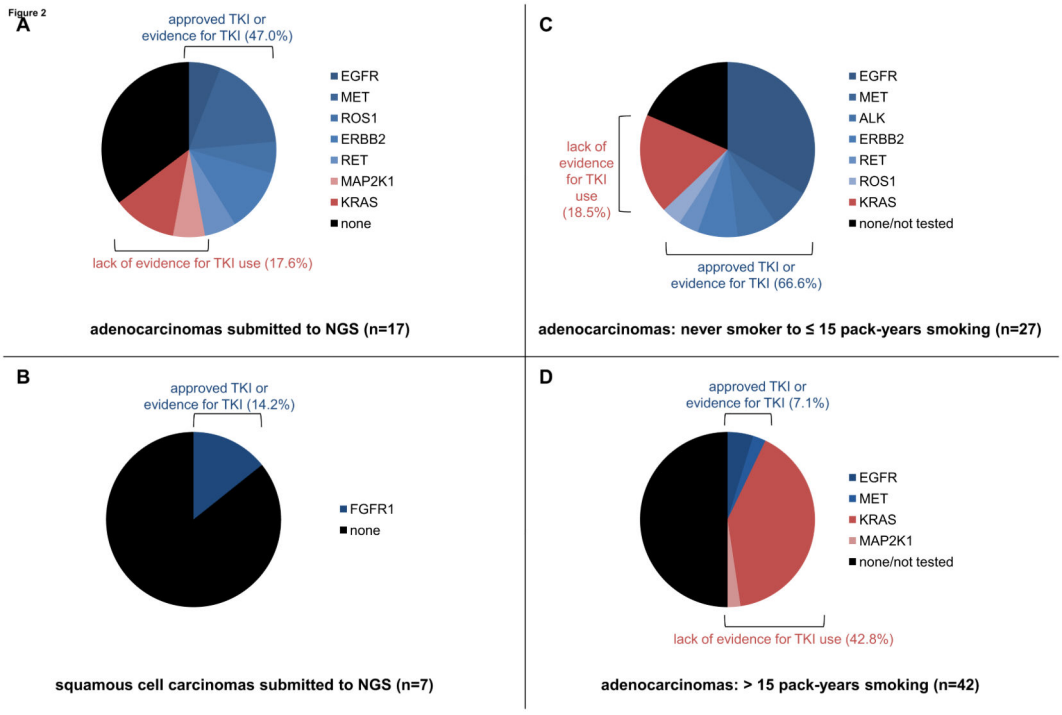

Figure 2.

Genomic abnormalities stratified by histology and patient smoking status. A.) Genomic findings and targeted therapies in adenocarcinomas, as identified by next generation sequencing (NGS). B.) Genomic findings and targeted therapies in squamous cell carcinomas, as identified by NGS. C.) Genomic findings and targeted therapies in adenocarcinomas from never-/light- smokers [≤ 15 pack-years smoking]. D.) Genomic findings and targeted therapies in adenocarcinomas from patients with > 15 pack-years smoking.

Table 1

Baseline characteristics of patients and tumors genotyped over a 6-month period.

Age at time of tissue acquisition Median (range)	67 (34-92)
Women n (%) Men n (%)	49 (59.7) 33 (40.3)
Race n (%) White Asian Black Other	 66 (80.5) 8 (9.8) 5 (6.1) 3 (3.6)
Smoking status n (%) Current smoker Former smoker Never smoker	 27 (32.9) 41 (50.0) 14 (17.1)
Stage n (%) I-III IV/recurrent	 23 (28.0) 59 (72.0)
Histology n (%) Adenocarcinoma Squamous cell carcinoma NSCLC (NOS)	 74 (90.2) 7 (8.5) 1 (1.2)
Type of tissue n (%) Surgical specimen Small biopsy Cytology block from aspirate/fluid	 22 (26.8) 17 (20.7) 43 (52.4)
Anatomic site of tissue acquisition n (%) Bone Brain Extra-thoracic lymph node Lung Mediastinal/hilar lymph node Pleura Other	 4 (4.9) 3 (3.6) 5 (6.1) 27 (32.9) 24 (29.3) 12 (14.6) 7 (8.5)

NSCLC, non-small-cell lung cancer; NOS, not otherwise specified

Table 2

Clinicopathologic characteristics, next generation sequencing (NGS) results and changes in clinical decisions for cases with successful assays

Patient no.	Stage/Sex/Race Smoking/pack-years	Single gene assay/results	Prior therapy	NGS assay	"Major" driver(s)	"Other" change(s)	Change in clinical decision	Clinical decision from NGS results
Adenocarcinomas								
1	IV/F/Asian never/0	No/ NA	Palliative radiotherapy	SNaPshot NGS V1 + gene fusion assay	EGFR p.delE746_A750	none	Yes	Consented first line erlotinib (clinical trial NCT00997334)
2	IV/F/Black never/0	Yes/ EGFR, ALK, KRAS, ROS1 negative	Palliative radiotherapy	SNaPshot NGS V1	ERBB2 p.E740_A741insAYVM	TP53 p. R342*	Yes	Consented clinical trial neratinib (NCT01827267)
3	IV/F/White former/5	Yes/ EGFR, ALK, KRAS negative; ROS1 failed	Palliative radiotherapy 1st line platinum-pemetrexed	SNaPshot NGS V1 + gene fusion assay	ERBB2 p.V747_G748insGSP	TP53 p.Y220C	Yes	Off label use afatinib
4	recurrent/M/White former/4	Yes/ EGFR, ALK, KRAS negative; ROS1 failed	Surgery Adjuvant platinum-docetaxel Palliative radiotherapy 1st line platinum-pemetrexed	SNaPshot NGS V1 + ALK, RET, ROS1 NGS gene fusion assay	EZH-ROS1 fusion	none	Yes	Recommended clinical trial crizotinib (NCT00585195) at progression; not consented
5	IV/F/Asian never/0	Yes/ EGFR, ALK, KRAS negative; ROS1 not performed	1st line platinum-paclitaxel 2nd line docetaxel	Foundation One	KIF5B-RET fusion	PTEN_D326fs*18	Yes	Consented clinical trial sunitinib (NCT01829217)
6	IV/F/White former/12	Yes/ EGFR, ALK, KRAS, ROS1 negative	1st line platinum-pemetrexed	Foundation One	MET amplification	GRIN2A p.F183I KDM5C p.P380fs*50 PBRM p.R1010* NKX2-1 amplification	Yes	Consented clinical trial crizotinib (NCT00585195)
7	IV/F/White former/24	Yes/ EGFR, ALK, KRAS, ROS1 negative	Palliative radiotherapy 1st line platinum-pemetrexed	SNaPshot NGS V1 + gene fusion assay	MET p.D1010_splice exon 14 PIK3CA p.E542K	TP53 p.R337L	Yes	No immediate change in therapy. Consideration of off label crizotinib at progression
8	IV/M/White former/16	Yes/ EGFR, ALK, KRAS	Palliative radiotherapy	SNaPshot NGS V1	KRAS p.Q61H	none	Yes	No immediate change in therapy. Consideration of clinical trial selumetinib (NCT01933932) at

Patient no.	Stage/Sex/Race Smoking/pack-years	Single gene assay/results	Prior therapy	NGS assay	"Major" driver(s)	"Other" change(s)	Change in clinical decision	Clinical decision from NGS results
		negative; ROS1 failed	1st line platinum-pemetrexed					progression
9	IV/F/White former/30	Yes/ EGFR, ALK, negative; KRAS p. G12C; ROS1 not performed	Palliative radiotherapy 1st line platinum-paclitaxel 2nd line pemetrexed	SNaPshot NGS V1	KRAS p.G12C	TP53 p.A159P	No	No clinical recommendation
10	IV/F/White former/25	Yes/ EGFR, ALK, KRAS, ROS1 negative	1st line platinum-pemetrexed 2nd line docetaxel	SNaPshot NGS V1	MAP2K1 p.K57N	STK11 p.T308C	No	No clinical recommendation
11	II/F/White never/0	Yes/ EGFR, ALK, KRAS, ROS1 negative	Surgery Adjuvant platinum-pemetrexed	SNaPshot NGS V1 + gene fusion assay	MET p.D1010N _splice exon 14	none	No	No clinical recommendation
12	IV/F/White never/0	Yes/ EGFR, ALK, KRAS, ROS1 negative	1st line platinum-paclitaxel 2nd line docetaxel 3rd line pemetrexed	SNaPshot NGS V1	none	MET p.D981V GNA11 p.L229F	No	No clinical recommendation
13	IV/F/White current/40	Yes/ EGFR, ALK, KRAS, ROS1 negative	Best supportive measures	SNaPshot NGS V1	none	TP53 p.G226*	No	No clinical recommendation
14	recurrent/M/White former/40	Yes/ EGFR, ALK, KRAS, ROS1 negative	Surgery	SNaPshot NGS V1	none	CDH1 p.D805N CDKN2A p.H123Q	No	No clinical recommendation
15	IV/M/White current/50	Yes/ EGFR, ALK, KRAS negative; ROS1 failed	1st line platinum-paclitaxel 2nd line docetaxel	SNaPshot NGS V1	none	TP53 p.F134L MET p.T992I SMAD4_ splice	No	No clinical recommendation
16	II/M/Asian current/10	Yes/ EGFR, ALK, KRAS, ROS1 negative	Surgery	SNaPshot NGS V1	none	TP53 p.R273C	No	No clinical recommendation
17	II/M/White former/40	Yes/ EGFR, ALK,	Surgery	SNaPshot NGS V1	none	TP53 p.R273L STK11_splice	No	No clinical recommendation

Patient no.	Stage/Sex/Race Smoking/pack-years	Single gene assay/results	Prior therapy	NGS assay	"Major" driver(s)	"Other" change(s)	Change in clinical decision	Clinical decision from NGS results
		KRAS, ROS1 negative				FGFR1 p.D132A (SNP)		
Squamous cell carcinomas								
18	III/F/White/ former/40	No/ NA	Definitive radiotherapy concurrent platinum-paclitaxel	SNaPshot NGS V1 + FGFR1 FISH	FGFR1 amplification (borderline)	TP53 p.R283P	No	No clinical recommendation
19	Recurrent/M/White former/60	No/ NA	Surgery Palliative radiotherapy	SNaPshot NGS V1 + FGFR1 FISH	none	TP53 p.R181P STK11 p.G187Vfs*	No	No clinical recommendation
20	IV/M/White former/80	No/ NA	Palliative radiotherapy 1st line platinum-paditaxel	SNaPshot NGS V1 + FGFR1 FISH	none	TP53 p.L130V	No	No clinical recommendation Subsequently consented clinical trial docetaxel (NCT01750281)
21	IV/M/White current/50	No/ NA	Palliative radiotherapy 1st line platinum-paclitaxel	SNaPshot NGS V1 + FGFR1 FISH	none	TP53 p.P36Afs*7 APC p.T1218M NOTCH1 p.Q1476S	No	No clinical recommendation
22	IV/M/White current/90	No/ NA	1st line platinum-paclitaxel	SNaPshot NGS V1 + FGFR1 FISH	none	TP53 p.L257G	No	No clinical recommendation
23	IV/M/White never/0	Yes/ EGFR, ALKnegative; KRAS, ROS1 not performed	1st line platinum-gemcitabine 2nd line docetaxel	SNaPshot NGS V1 + gene fusion assay	none	TP53 p.delV157_ M160	No	No clinical recommendation
24	I/M/White former/30	No/ NA	Surgery	SNaPshot NGS V1	none	TP53 p. H178D	No	No clinical recommendation